Journal of Chromatography, 547 (1991) 468–471
Elsevier Science Publishers B.V., Amsterdam

CHROM. 23 254

Short Communication

Analysis of some tropane alkaloids in plants by mixed-column high-performance liquid chromatography[a]

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(First received September 3rd, 1990; revised manuscript received February 25th, 1991)

ABSTRACT

A simple and rapid high-performance liquid chromatographic method for the determination of atropine and scopolamine in plants using the combination of two different polarity columns in series and direct injection of plant extract is described. Application of the method to the analysis of two species of solanaceous plants is reported.

INTRODUCTION

The analysis of tropane alkaloids in solanaceous plants is of interest because of the extensive use of atropine and scopolamine in pharmaceutical preparations. Using an ion-pair technique, the analysis of tropane alkaloids by high-performance liquid chromatography (HPLC) has been reported [1–7]. Anetai and Yamagishi [8] applied an HPLC method which was not an ion-pair technique to analyse crude drugs from plants, but the method is only suitable for atropine because the scopolamine peak overlaps the impurities peak. A combination of columns using sodium acetate buffer has been applied for the analysis of atropine and its degradation products [9].

The aim of the present study was to explore the chromatographic possibilities of using a combination of two different polarity columns and a non-ion-pair technique in an attempt to overcome difficulties associated with co-elution of scopolamine and impurities in the analysis of plant extracts. The use of buffer was avoided, as constant use of salts when there is a large number of samples to be analysed creates the problem of corrosion and excessive wear of the pump piston and seal, which can cause leaks [10]. A simple method for determination of atropine and scopolamine in solanaceous plants using a combination of HPLC columns employing acetonitrile–water, containing 0.5% triethylamine as the mobile phase, was determined.

[a] CIMAP publication No. 1011.

0021-9673/91/$03.50

EXPERIMENTAL

Reagents

The reagents used were HPLC-grade acetonitrile and triethylamine. Water was deionized and double-distilled in glass. All the solvents and solutions were filtered through a Millipore filter. Atropine and scopolamine hydrobromide were obtained from Sigma (St. Louis, MO, USA).

Apparatus

A Waters modular HPLC system, consisting of a U6K injector, M-6000A pump, M-450 variable-wavelength detector and M-730 data system, was used. Analyses were performed on RP-C_{18} and RP-CN columns (150 × 3.9 mm; particle size 5 μm) obtained from Waters (Division of Millipore, Milford, MA, USA). The combination was made by connecting the outlet of RP-CN to the inlet of RP-C_{18}.

HPLC conditions

The composition of the mobile phase was optimized by varying the percentage of acetonitrile to give the operating conditions as follows: acetonitrile–water (35:65) containing 0.5% triethylamine, flow-rate 0.8 ml/min, column temperature 30°C, detector wavelength 254 nm and detector sensitivity 0.04 a.u.f.s.

Calibration graphs

Known amounts of atropine and scopolamine free bases were dissolved in methanol to give corresponding standard solutions of concentration 2 mg/ml. Different volumes of these standards were processed using the HPLC conditions described above. The area counts of peaks and the corresponding concentrations were used to construct the calibration graphs. The graphs followed Beer's law in the range 1–50 μg. The regression equations for atropine and scopolamine are $y = 0.3190\ x + 0.0099$ ($r = 0.998$) and $y = 0.4480\ x + 0.1599$ ($r = 0.999$), respectively.

Extraction procedure

Extraction of crude alkaloids from different parts (inflorescence, leaf and stem) of *Hyoscyamus muticus* and *Hyoscyamus niger* was carried out using the following method [11]. Dried powder (10 g) of plant material was extracted with 200 ml of solvent (dichloromethane–methanol–ammonium hydroxide, 70:25:5, v/v/v) for 2 h and filtered. The filtrate was extracted with 100 ml of 0.5 *M* sulphuric acid. The aqueous layer was separated and basified with ammonium hydroxide. The basified solution was extracted with dichloromethane (4 × 80 ml) and evaporated to dryness to give crude alkaloids, which were dissolved in 10 ml of methanol. Samples were filtered through a Millipore sample filtration kit. A 2–10 μl sample of this extract was used for injection into the HPLC column.

RESULTS AND DISCUSSION

The number of theoretical plates (N) calculated for atropine and scopolamine on different columns and their combinations is given in Table I. The maximum value of N for scopolamine was achieved only on the combination RP-CN + RP-C_{18}. In

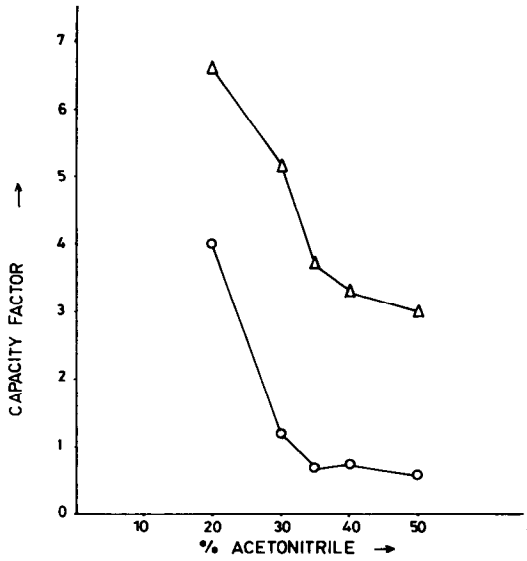
TABLE I

NUMBER OF THEORETICAL PLATES (N) OF DIFFERENT COLUMNS AND COMBINATIONS

Dimension of columns: 150 × 3.9 mm; particle size 5 μm.

Alkaloid	N			
	C_{18}	CN	C_{18} + CN	CN + C_{18}
Atropine	1524	1542	4045	2896
Scopolamine	506	360	747	1304

fact it is the scopolamine peak that needs higher resolution to overcome the problem of co-elution with impurities in plant extract, and atropine has no such difficulties. This combination provided reproducible and accurate results with good resolution of atropine and scopolamine in plant extract without interference from impurities. The order of the columns is very significant because reversal of the sequence results in very poor resolution and overlapping of the scopolamine peak with impurities. The graph in Fig. 1 shows the capacity factor (k') *versus* percentage of acetonitrile in the mobile phase. The resulting chromatogram of atropine and scopolamine using the HPLC conditions given above is shown in Fig. 2. Quantitative results of individual alkaloids in various parts of *Hyoscyamus muticus* and *Hyoscyamus niger* are reported in Table II. The standard deviation and coefficient of variation for atropine are 0.57 and 2.94,



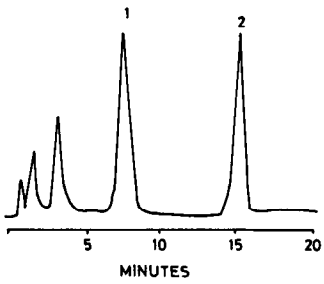


Fig. 1. Effect of acetonitrile concentration in the mobile phase on capacity factor. Column combination: RP-CN and RP-C_{18}. ○ = Scopolamine; △ = atropine.

Fig. 2. Resolution of atropine and scopolamine. Column combination: RP-CN and RP-C_{18}. Mobile phase: acetonitrile–water (35:65) + 0.5% triethylamine; amount of alkaloid 10 μg each. Peaks: 1 = scopolamine; 2 = atropine.

TABLE II

CONTENT OF ATROPINE AND SCOPOLAMINE IN DIFFERENT PARTS OF PLANTS

Sample	Atropine			Scopolamine		
	Content (%)	S.D. (%)	C.V. (%)	Content (%)	S.D. (%)	C.V. (%)
Hyoscyamus muticus						
Inflorescence	0.720	2.5	3.4	0.043	0.20	4.6
Leaf	0.615	1.6	2.6	0.002	0.02	8.0
Stem	0.341	1.0	2.9	–	–	–
Hyoscyamus niger						
Inflorescence	0.025	0.10	4.0	0.040	0.20	5.0
Leaf	0.010	0.06	6.0	0.035	0.12	3.5
Stem	0.020	0.11	5.5	0.032	0.15	4.7

respectively, and for scopolamine are 0.39 and 1.97, respectively.

Because of its simplicity, selectivity and accuracy, the HPLC assay described here is suitable for the analyse of tropane alkaloids in plants extracts.

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